II. RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 1-25 and 39 are canceled. New claims 40 and 41 have been added. Claim 40 is basically a re-write of previous claim 25 in a more understandable fashion to address the enablement concerns. However, it should be noted that in light of the newly identified evidence of non-obviousness, claim 40 has been broadened and is no longer limited to human antibody production. This element has been moved to new claim 41. Dependent claims 26, 31-34 and 37-38 have been amended to correct their dependency to depend from new independent claim 40. Claims 26-38, 40 and 41 are pending and under examination.

B. Claim Rejections – 35 U.S.C. §112, First Paragraph

Applicants have read the rejection and, unfortunately, do not fully understand or appreciate the basis for the rejection. The Action states that the specification is enabling for generating antibody-producing cells from a Xenomouse/Immortomouse cross, where the antigen is presented to the mouse *in vivo*, but alleges that the specification is not enabling for removing cells from the mouse and immunizing them *in vitro*. Notably, there is no reasoned explanation as to ***why*** the specification is not enabling. Moreover, it is noted that on page 4 of the Action, the Examiner concedes that "[o]ne of skill would readily recognize that antibodies could be produced by simply contacting a cell capable of producing antibodies with an antigen *in vitro*." Perhaps this latter statement is a typo. If so, the Examiner is requested to clearly note for the record that one of skill would ***not*** readily recognize that antibodies could be produced by simply contacting a cell capable of producing antibodies with an antigen *in vitro*.

Nevertheless, regardless of what one of skill would or would not recognize upon reading the present specification, we would note that working Example 1 presents actual studies demonstrating the successful *in vitro* immunization of spleen cells and their subsequent immortalization without hybridoma formation. With this evidence, the Examiner will have to present some cogent explanation of the enablement rejection in order to maintain the rejection. See MPEP 2164 *et seq*.

C. Claim Rejections -- Obviousness

The Examiner has maintained an obviousness rejection with respect to claims 25-38 over Green, Jat *et al.* ("Jat"), Kano and Lidington *et al.* ("Lidington"). We respectfully traverse, for the following reasons.

While Green does teach a mouse that is capable of producing human antibodies (the so-called Xenomouse®), as recognized by the Examiner, the cells of the Green mouse are ***incapable*** of producing immortalized populations of antigen-producing cells without forming hybridomas. Indeed, as the Examiner recognized in previous Actions, Green teaches that the Xenomouse® is to be used in "established hybridoma procedures (i.e., immortalization) to produce monoclonal antibodies." Action of 12/8/06 at p. 8. Thus, Green actually requires that one proceed through hybridoma production in order to produce humanized antibodies (see, *e.g.*, p. 13 ("The use of Xenomouse mice in conjunction with well-established hybridoma procedures ...") and p. 18 ("Generation of hybridomas followed well-established procedures...")). Indeed, no other alternative to producing immortalized populations of antibody-generating cells ***other*** than through hybridoma generation is contemplated by Green.

Turning to Kano, it is noted that Kano specifically teaches that the immortalizing oncogene is introduced into the immunized ***rabbit*** spleen cells ***ex vivo*** – after the spleen has been

removed from the immunized rabbit – which again is clearly contrary to the present invention, wherein the spleen cells will inherently have this capability without *ex vivo* introduction of an oncogene. Moreover, the Examiner has failed to explain how one of skill would combine genetic elements of a mouse with those of a rabbit.

Turning to Jat, the Action appears to concede that Jat is silent regarding the possibility of further modifying the Jat Immortomouse to include the genetic components for human antibody production. We would note that there is simply no basis from Jat that would provide any suggestion, motivation or reasonable expectation of success that such a mouse should or even could be prepared and that such a mouse would be capable of producing human antibody producing cells that are capable of being immortalized.

Further, we note that Jat discloses the immortalization of only a very few cell types from the Immortomouse, including only skin fibroblasts and thymic epithelial cells, neither of which are antibody producing cells. Thus, since Jat is silent with respect to antibody producing cells, there is no reason that one of skill reading Jat would choose to genetically engineer an Immortomouse to incorporate human antibody genes.

Indeed, Jat clearly stands for the further proposition that there is a high degree of uncertainty regarding the ability to immortalize individual cell types from such a mouse. For example, Jat notes that the ability to immortalize thymic epithelial cells as compared to that of skin fibroblasts varied substantially, concluding, for example, that "[t]he different effect of the transgene on thymus and liver in vivo suggest that cell types can differ in their susceptibility to the action of Tag" (see paragraph bridging pages 5099/5100 and first paragraph page 5100). Jat reinforces this uncertainty in the final paragraph, stating that having the Immortomouse "will ***allow us to determine whether this approach*** to cell line production is applicable to tissues—

including embryonic tissues—other than skin and thymus...[and]...***may*** allow direct derivation of cell lines from a wide variety of different tissues and cell types" (emphasis ours). At best, Jat can be said to merely offer an invitation to experiment, with no reasonable expectation of success.

Lidington does not appear to be in any way relevant, as this reference is concerned with the immortalization of growth factor-responsive cardiac endothelial cells. The Action makes reference to Lidington's reference to intercrossing to introduce ts58A into the genetic background of mice. Action, top of page 7. However, the ***complete*** excerpt from Lidington continues by stating that such intercrossing is to "provide a ready source of EC [endothelial cells] from a variety of different vascular beds for further study, thus enhancing our understanding of the role of the endothelium in the pathophysiology of cardiovascular tissue." Clearly, Ludington is concerned only with epithelial cells and says nothing about crossing to introduce a human antibody genetic background or the preparation of immortalized antibody-producing cells.

The Examiner postulates that someone reading Green would somehow be motivated to proceed in a manner ***contrary*** to Green's teaching – by crossing Green's Xenomouse with Jat's Immortomouse. We would again note that the only method taught in Green for producing human monoclonal antibodies is to proceed through the "established hybridoma procedure" using immortalization. However, the claims at issue require that such is achieved "without forming hybridomas." Hence, Green actually teaches away from the solution provided by the present invention. See *In re Hedges*, 228 USPQ 685, 687 (Fed. Cir. 1986) (Proceeding contrary to the accepted wisdom of the prior art is strong evidence of non-obviousness.) Indeed, we submit that the Examiner conjecture, taken out of thin air, that someone skilled in the art would

be somehow be motivated by Green to proceed in a manner contrary to Green's teaching is the worst form of hindsight analysis – an approach forbidden by the case law. *In re Dow Chemical Co.*, 5 USPQ2d 1529 (Fed. Cir. 1988).

Most importantly, though, there is simply no basis on this record for concluding that there was a reasonable expectation of success. Such reason are inherent in the Examiner's enablement rejections – frankly, one of skill would not have had a reasonable expectation that they could i) successfully prepare a mouse that stably contains the genetic elements for both human antibody production and immortalization, and ii) successfully immortalize antibody producing cells, much less, antibody producing cells that contain transgenic human genes for antibody production.

Applicants enclose the declaration of the inventors Drs. Wadih Arap and Renata Pasqualini, in support of a conclusion of non-obviousness of the present invention. As stated by Drs. Pasqualini and Arap, they are giving this declaration to provide evidence that the invention disclosed and claimed in the subject application would not have been obvious to a person of ordinary skill in the art in that such a person would have had no reasonable expectation, *a priori*, that immortalization of splenocytes obtained from mice having the genetic components for human antibody production as well as the genetic components for immortalizing cells would actually be successful.

They first observe that a preferred aspect of the invention involves the use of a mouse whose genome includes the genetic components for human antibody production as well as the genetic components for immortalizing cells, such as the temperature-sensitive SV40 Large Tumor antigen (tsSV40Tag). The continue that in order to maintain cells from such a mouse in a conditionally "immortal" state, it is required to maintain them at a lower temperature, such as around 33° C, and that it is not at all a given that the proliferation rates among spleen-derived

cells from such a mouse, upon plating at 33° C would allow for the successful expansion and selection of monoclonal splenocyte lines that secrete antibodies or interest at sufficient amounts. They observe that the spleen is a complex organ, which contains several cell types, including macrophages, fibroblasts, splenocytes, endothelial and stromal cells, T and B cells. Fibroblasts are well known to be among the most significant impediment for the establishment of primary cell lines, if they are present in a certain preparation that is plated under tissue culture conditions, in that they tend to take over the culture. The continue by stating that just among the antibody-secreting and non-secreting splenocytes derived from an H-2K^b-tsA58 transgenic mouse the expected outcome is that the non-secreting cells would grow much faster and take over the culture, preventing the growth of the secreting lines. Indeed, this very point was raised by a scientific referee (a member of the United States National Academy of Sciences and an editor of the *Proc. Natl. Acad. Sci. USA*) who evaluated our manuscript that describes our invention ("Hybridoma-free generation of monocloncal antibodies," *Proc. Natl. Acad. Sci. USA, 101*:257-259, 2004; see Exhibit 2 of the declaration). A copy of the referee's comments are attached as Exhibit 3 to the declaration.

Drs. Pasqualini and Arap continue by emphasizing that even conventional hybridoma production is quite problematic because in a random admixture of clones, non-secreting clones will overtake the antibody-secreting ones. This occurs since different proliferation rates that tend are slower in the antibody-secreting ones. As further pointed out by the referee, it was expected that, even if obtained, the cloning of monoclonal lines representing antibody-secreting cells would not be possible due to their rarity and inability to grow within the constrains of limiting dilution. Surprisingly, this turned out not to be the case as well, given that the tsSV40Tag spleen cell mixtures were found to provide an ideal system for single cell cloning, due to the presence of

other immortalized cells (such as macrophages, stromal cells, and endothelial cells, among others) that apparently serve as an intricate feeder layer. Thus, one of ordinary skill in the art, as perhaps exemplified by the referee, would not have had an expectation to believe *a priori* that immortalization of splenocytes obtained from back-crossings of H-2K^b-tsA58 mice and transgenic mice containing a replaced genetic complement for human antibody production would actually be successful. Indeed, as can be seen from Exhibit 3, the National Academy referee specifically stated that this achievement was considered surprising.

Thus, for the foregoing reasons it is submitted that the Examiner has failed to set forth a *prima facie* case of obviousness and Applicants thus respectfully request that the rejection be withdrawn.

CONCLUSION

Applicants believe that the foregoing remarks fully respond to all outstanding matters for this application. Applicants respectfully request that the rejections of all claims be withdrawn so they may pass to issuance.

Should the Examiner desire to sustain any of the rejections discussed in relation to this Response, the courtesy of a telephonic conference between the Examiner, the Examiner's supervisor, and the undersigned attorney at 512-536-3055 is respectfully requested.

Respectfully submitted,

46,955

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